FINAL REPORT

WTFRC Project #AE-01-51

WSU Project # 4094

Project title:	Monitoring and control of stink bugs
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Objectives:

- Examine candidate insecticides for stink bug control in-orchard.
- Examine effects of pyrethroid insecticides on non-target organisms (mites).
- Re-evaluate the potential of pheromone based aggregate-and-kill strategies to manage stink bugs before mating and oviposition in late spring/early summer with larger blocks.
- Determine daily phenology of stink bug activity.
- Re-examine attractive radius of pheromone and continue dose/response studies to find optimum release rate for stink bug lures.
- Determine the potential for increasing the attractiveness of pheromone lures with the addition of feeding stimulants/arrestants.

Significant findings:

1999

- Removal of native vegetation from borders did not reduce damage by stink bugs in border rows.
- Baiting native vegetation with aggregation pheromone adjacent to orchard borders did not reduce fruit damage by stink bugs in border trees.
- Orthene appeared to offer the best suppression of stink bugs in borders.
- Three dispensers containing the major component of stink bug pheromone were evaluated for attractiveness to stink bugs. All lures attracted aggregations of stink bugs to mullein plants, but the same lures placed inside different traps were not effective.
- Fruit in "trap trees" placed in orchard borders had more damage than fruit in adjacent trees or control trees without lures.
- Additional pheromone components provided by UC Riverside did not increase aggregations of the consperse stink bug.
- The impact of Surround (kaolin) treatments to orchard borders in reducing fruit injury was highly variable. Fruit damage was significantly higher above 6 feet than below, and there was a strong border effect (70% of damage in the first two rows) in almost all orchards where studies were conducted.
- Apple alone does not provide a sufficient nutrient source to mature stink bugs.
- Feeding damage from stink bugs is evident within 24-48 hours following feeding.

2000

- Mark-recapture studies demonstrated that the pheromone is fairly short range, with an active space of 10-25 m. These studies also provided estimates of the numbers of bugs present in areas bordering orchards.
- Pheromone release devices were tested at a range of release rates. These studies, performed in cooperation with Dr. Peter Landolt, demonstrated that it is possible to attract stink bugs at rates

far below what we are currently using. Lower rates may also enhance trap catch by providing a more "realistic" stimulus to induce bugs to enter traps.

- Research conducted in conjunction with Dr. Jocelyn Millar (UC Riverside) in search of a generic lure revealed that there is no inhibition of attraction of either of the two major stink bug species in our area (*Euschistus conspersus* and *Chlorochroa ligata*) when the pheromones of both species are present on the same plant.
- A blend of "green" chemicals common to a variety of host plants, including alfalfa, was field tested for attractiveness. The tested blends did not increase attraction of stink bugs to baited plants.
- A survey of parasites impacting stink bugs in central Washington indicated that two species of parasitic fly (Tachinidae; *Gymnoclytia occidentalis* and *Gymnosoma filiola*) and two species of parasitic wasp (Scelionidae; *Trissolcus utahensis*, *Trissolcus euschisti*, and *Telenomus podisi*) were identified. Parasitism and predation rates were assessed throughout the season, with predation being more important.
- Surveys conducted in the Columbia Basin determined that the stink bug complex in this area differed when compared to the complex in north-central Washington. Far lower numbers of *E. conspersus* were observed which may partially explain the lower level of stink bug damage experienced by the majority of Yakima area growers.
- Analysis of the surfaces of the beaks, or stylets, of *E. conspersus* collected in a variety of orchards from Orondo to Manson consistently revealed the presence of a single species of fungus. This fungus was isolated in pure culture and identified. If this fungus is pathogenic to apple, it may be a significant factor in the visible appearance of damage.
- Stink bug eggs were caged on apple limbs and they were unable to complete development on apple alone.
- Male stink bugs are sexually mature upon emergence from overwintering sites (early April), but females mature approximately two weeks after emergence. This information can be used to time mating, egg laying and subsequent control measures.

2001

- A number of insecticides were assayed in 2001 and the pyrethroid Danitol emerged as the clearly superior compound for stink bug control. However, this compound caused a reduction in populations of predatory mites and therefore has the potential to "flare" pest mite species. These data will be collected in spring 2002 to determine any carry-over effects of late summer and fall Danitol applications.
- Previous border baiting and spraying efforts succeeded in attracting large numbers of bugs to mullein plants, but the insecticide used (imidacloprid) had poor efficacy. This year's results using a more effective insecticide (Carzol) were far more encouraging, as damage in treated areas was significantly reduced compared with check plots.
- Pheromone release rate studies revealed an optimum release rate, and this information may be used to determine the best release device for commercialization of the pheromone.
- A number of experiments involving videotaping of stink bugs on baited and unbaited host plants were conducted to further quantify stink bug behavior. Much of the stink bug movement to pheromone sources and mating occurs at night. These data are valuable from a management perspective (timing of sprays), as well as monitoring (timing of examination of baited plants).
- We identified a novel stink bug feeding stimulant. This compound induced stink bugs to feed and to remain in contact with treated areas longer than untreated areas. Combination of this compound in solution with the insecticide Danitol did not result in a significant decrease in the attractiveness of the compound. These data open the possibility of using this compound to enhance insecticide efficacy or possibly incorporating it into bait stations.

Results and Discussion - 2001

Chemical screening: The insecticides listed in Table 1 were assayed for activity against *E. conspersus.* These compounds represent a variety of old and new chemistries and were chosen based on a variety of factors including availability, re-entry interval and effects on non-target organisms. Table 1 shows acute toxicity of the compounds using a Potter tower assay. Compounds that demonstrated efficacy in Potter tower tests were applied to mullein leaves, which were then allowed to age in the field for 48 h before stink bugs were exposed to the leaves in a Petri dish. Results of this leaf disc bioassay are shown in Table 2. In both testing methods the pyrethroid insecticide Danitol was the most effective, followed by Thiodan and Swat (phosphamidon). Carzol and Thiodan are currently the recommended in-orchard treatments for stink bug control; however, Thiodan has a long PHI and restrictions on the number of applications per year, and Carzol can only be applied once after bloom and then under prescription use regulations. Both of these products could be phased out in the future due to FQPA action. Danitol is labeled for apple and appears to provide growers with an effective alternative for control of stink bugs.

Treatment	Rate	Rate	Corrected % mortality			
	(ppm)	(Form./100 gal)	24 h.	48 h.	96 h.	1 week
Assail 70WP	60	1 oz.	4	4	4	46
Avaunt 30WDG	34	1.5 oz.	0	0	0	0
Carzol 92SP	413	6 oz.	12	35	63	74
Danitol 2.4 EC	106	4.8 fl. oz.	98	98	98	98
Swat 8 EC	300	4 fl. oz.	90	95	97	100
Thiodan 50WP	149	1 lb.	12	42	59	91
Warrior T 1SC	7	1 oz.	64	96	96	96

Table 1. Corrected percent mortality of stink bugs exposed to insecticides applied in Potter tower bioassay.

Table 2.	Corrected percent mortality of stink bugs exposed to insecticides applied to mullein leaf
	discs.

Treatment	Rate	Rate	Corrected % mortality			
	(ppm)	(Form./100 gal)	24 h.	48 h.	72 h.	120 h.
Carzol 92SP	413	6 oz.	12	12.5	16	16
Danitol 2.4 EC	106	4.8 fl. oz.	76	84	89	92
Swat 8 EC	300	4 fl. oz.	32	50	100	100
Thiodan 50WP	149	1 lb.	84	84	100	100
Warrior T 1SC	7	1 oz.	16	21	21	23

Danitol – **In-orchard efficacy:** Danitol sprays were applied on August 13, 2001, to all orchards in the study (n=7). This date was chosen because the second, or summer, generation of stink bugs had reached adulthood and the onset of fruit damage had been noted. The purpose of our study was to determine the in-orchard effects of Danitol in reducing stink bug injury and to assess the impact of treatments on spider mites and predatory mites. The results, summarized in Fig. 1, were quite encouraging from a management standpoint, reducing damage by over 80% on average (72-90%). It should be noted that these are extremely high-pressure orchards - in many cases 100% of the fruit on the border row were damaged. The reduction in fruit damage was achieved with only a single Danitol application, and the timing of this spray could likely have been improved (i.e., earlier in season). It is also important to note that the sprays were applied at, or shortly after, dusk – the period of highest stink bug activity.

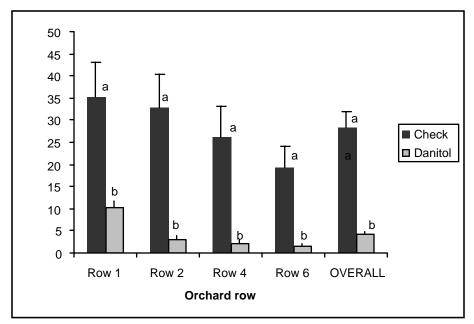


Figure 1. Stink bug fruit damage at harvest following a single in-orchard application of Danitol at label rate (20 fl. oz/acre) on August 13, 2001 (n=7 orchards). Bars with same letter superscript in each category are not significantly different (P<0.05).

Danitol – **Non-target effects:** The potential of Danitol as a powerful tool against stink bug populations in orchard led us to investigate its effects on non-target mites. Pyrethroid insecticides, such as Danitol, have been shown to cause increases in populations of pest mite species. We therefore conducted pre- and post-counts of European red mite (ERM), twospotted spider mite, apple rust mite, and beneficial mite populations (*Typhlodromus* and *Zetzellia* species) in six commercial orchards where Danitol had been applied at the label rate of 21 fl. oz./acre. The results are summarized in Table 3. Significant differences in mite densities are denoted by alphabetic superscripts where means within each column followed by different letters are significantly different.

Densities of both spider mites and beneficial mites were impacted by the Danitol treatment. However, it is important to note that beneficial mite populations often have a lag time in recovering from exposure to pyrethroids, and the full effects of these insecticide treatments may not be seen until next spring. We will be conducting surveys in the spring of 2002 to determine whether beneficial mite populations show long-term effects of Danitol exposure.

lintes/30 leaves)						
Spider mites*			Benet	ficials*	Ratio	
Date	Egg	Motile	Rust	Egg	Motile	ERM/beneficials
Pre-count - 8/13/01						
Treated	12.1^{a}	2.3 ^a	10.1^{a}	0.28^{a}	0.96^{a}	2.75:1 ^a
Check	18.5^{a}	$2.7^{\rm a}$	6.8^{a}	0.64^{a}	1.24 ^a	$1.84:1^{a}$
24-h post - 8/21/01						
Treated	6.1 ^a	0.5^{a}	42.04 ^a	0.02^{a}	0.04^{a}	9:1 ^a
Check	13.6 ^a	8.8^{b}	71.84 ^a	$0.04^{\rm a}$	0.36^{b}	18:1 ^b
8-wk post - 10/11/01						
Treated	2.2 ^a	$0.8^{\mathbf{a}}$	55.28 ^a	0	0^{a}	N/A
Check	10.8 ^a	5.9 ^b	97.24 ^b	0	0.92^{b}	5.17:1

Table 3.	Counts of pest and beneficial mites in orchards pre- and post-Danitol treatment (# of
	mites/50 leaves)

* Counts of spider mites represent totals of European red mite and twospotted spider mites; counts of beneficials represent totals of Typhlodromus + Zetzellia spp., as the dominant species varied by locations

Bait-and-spray trials: Experiments conducted in 2000 revealed that stink bugs could be concentrated on orchard border plants (mullein) in large numbers using synthetic pheromone. However, our previous attempts to attract-and-kill the insects used Provado, which proved to be ineffective for stink bug control. Trials in 2001 used a more effective toxicant (Carzol). The protocol, the same as that used in 2000, was as follows: Beginning in mid-May (onset of mating/egg-laying for *E. conspersus*), aggregation pheromone lures were placed at 20-foot intervals on host plants (mullein). These baited plants were clearly flagged and remained constant throughout the season. Flagged plants were sprayed to drip using a handgun at weekly intervals throughout the entire egg-laying period (ending in early July). Four blocks were used in the study. Blocks were 400 feet in total length and were paired with 400-foot untreated sections of border.

Large numbers of bugs were attracted to treated plants during this period, and it was not uncommon to see 80 or even 100 bugs on a single mullein plant. Dead bugs were frequently noted beneath treated plants following insecticide treatments. Extensive sampling was conducted during the season following sprays (Fig. 1) and at harvest (Fig. 2), and these results are shown below.

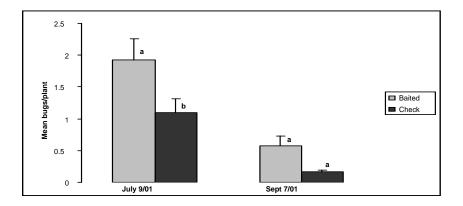


Figure 2. Mean bugs/plant at bait-and-spray and control (check) host plants in treatment areas at midand late season. Bars with same letter superscript at each date are not significantly different (P < 0.05).

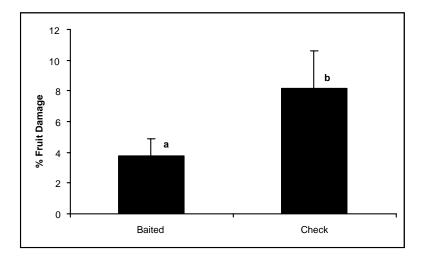


Figure 3. Mean stink bug damage to fruit at harvest on border rows associated with pheromone-baited vs. control border treatments (n=4). Bars with same letter superscript are not significantly different (P<0.05).

Stink bug chronology: Our observations of stink bug behavior have been restricted primarily to daylight hours when these insects were visibly active and moving among host plants, feeding, mating and laying eggs. It was assumed therefore that these insects were primarily active during daylight hours. However, an experiment filming bugs for 24 h in the field revealed that much of stink bug activity, particularly reproductive activity and response to pheromone, increased dramatically at dusk and continued through the hours of darkness. Bug counts at dawn were approximately half of those at their peak.

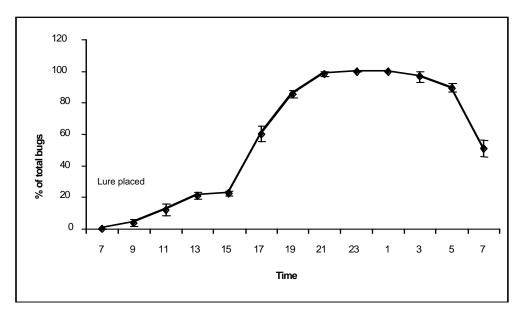


Figure 4. Twenty-four hour chronology of stink bug movement to baited host plants; lure was placed at 7:00 AM and removed 24 h later. Numbers expressed as a percentage of the total numbers of bugs visiting the plant in each case (n=3).

This suggests that our counts at baited lures may in fact be significantly lower than the peak catch of stink bugs. This also suggests that optimal spray timings may be in dusk, instead of the current

"default" spray timing of morning or mid-afternoon. Additional research in 2002 will investigate this behavior further as it relates to activities in the orchard, as our observations were conducted on mullein plants on the orchard border.

Active space of pheromone lures: We continued efforts undertaken in 2001 to determine the active space of pheromone lures for both monitoring and control efforts using mark-recapture studies. For each experiment, bugs were released at sites 5, 10 and 25 m from pheromone point sources. A known number of bugs was released at each location (150 bugs/location), with an equal number of males and females at each location. Bugs were marked on the thorax with nail polish to denote the different release distances. Surveys were conducted for eight days following release of bugs, with counts at the release area, the baited plant and various random plants in between. These counts revealed that bugs usually tended to move to the nearest mullein plant to their release area and begin feeding there. The majority of the bugs recaptured were from the 5 m release site (Table 4), suggesting that this pheromone is a short-range communication tool for these insects. This information confirms our observations of attraction in the field and will serve to give us an attractive radius for future trapping and attract-and-kill experiments.

	Overwintered generation adults		
Release distance	Total recaptured	% of total	
5 m	32	68	
10 m	10	21	
25 m	5	11	

Table 4.Results of mark-recapture study of stink bug adults,
showing numbers of bugs recaptured at baited mullein
plant from each of three release distances.

Dose/response experiments: Although the current 'WSU lure,' developed in 1999 and used extensively since, has been shown to reliably attract stink bugs in the field, it is not a practical long-term solution. The plastic caps are tedious to load, leaky, and require large amounts of material. Growers and consultants require an improved system of getting the pheromone to the field. In order to develop a commercial lure for stink bug monitoring and control, we continued studies initiated in 2000 with a variety of release rates. The rates were modified by placing equal volumes of pheromone inside polypropylene vials, then placing upon these vials lids with a variety of hole sizes in them. The hole size dictates the release rate of the pheromone (i.e., larger hole=higher release rate). The treatments ranged from included hole diameters of 0.02", 0.04", 0.063", 0.125", 0.25", and an undrilled vial (check). These lures were prepared by Dr. Peter Landolt at USDA-ARS Yakima.

We were able to find a release rate that did clearly out-perform the others tested (Fig. 5), and this release rate indicates that we are able to attract bugs with far less pheromone than we had previously been using. This translates into lower costs for manufacture of a commercial lure and greater flexibility in release devices, including the possibility of rubber septa.

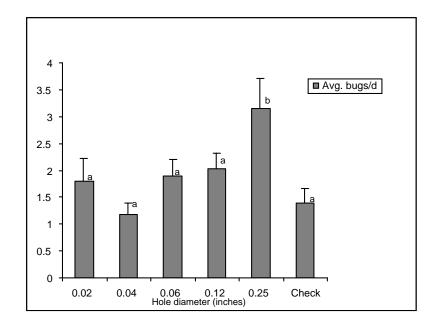


Figure 5. Mean bugs/day attracted to plants baited with synthetic pheromone at a range of release rates, determined by various hole sizes (n=25). Bars with same letter superscript are not significantly different (P<0.05).

Feeding attractant experiments: Experiments initiated early in 2001 revealed that a commercially available fertilizer product was very attractive to stink bugs in an aqueous solution. Where stink bugs were presented a choice between feeding on dental wicks soaked with either water only or a solution of the fertilizer in water, the fertilizer was shown not only to induce feeding but also stimulated bugs to feed longer than a water-only treatment (Fig. 6). This finding raises some possibilities in the realm of managing stink bugs using a bait-and-kill system, where a combination of the attractive pheromone, the fertilizer solution, and a killing agent (i.e., insecticide) could be used on bug-infested borders.

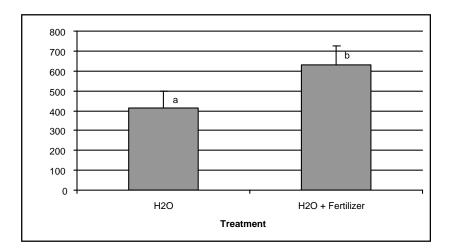


Figure 6. Time spent feeding by stink bugs in arena with 20-minute (1200 s.) cutoff point. Bars with same letter superscript are not significantly different (P<0.05)

Feeding attractant + **insecticide experiments:** Following the experiments described above, we initiated studies combining the attractive fertilizer with our most efficacious contact insecticide, the pyrethroid Danitol. The objective of the experiment was twofold: 1) to determine the minimal lethal dose of insecticide needed in solution; and 2) to determine whether there was an inhibitory or repellent effect upon bug feeding following addition of the insecticide. As in the previous experiment, the insects were presented with a choice test, and the time spent feeding (Fig. 7) and times until death (Fig. 8) were recorded. These data were recorded from a range of concentrations of Danitol with a check of fertilizer only. For reference, the field rate of Danitol results in a concentration of approximately 100 ppm.

The results of this experiment demonstrate that, while bugs did tend to feed upon the Danitol-treated wicks for less time than the control wicks, the exposure was still enough to cause death. These results are encouraging and suggest that the idea of a bait station that incorporates the attractive pheromone in combination with an insecticide/feeding attractant delivery system may be worth pursuing further.

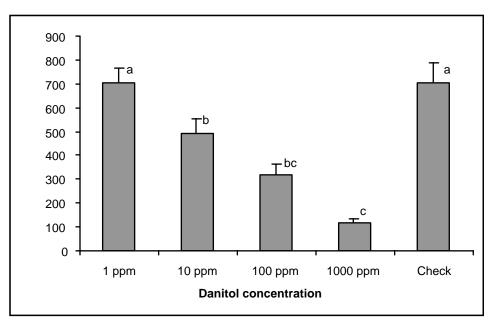


Figure 7. Time spent feeding upon cotton wicks soaked with fertilizer and varying concentrations of Danitol (n=30). Bars with different letter superscripts are not significantly different (P<0.05).

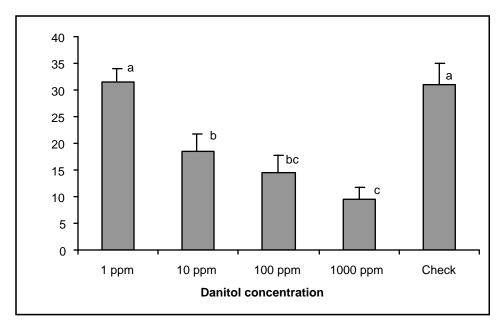


Figure 8. Time elapsed until death of bugs following feeding upon cotton wicks soaked with fertilizer and varying quantities of Danitol (n = 30). Bars with different letter superscripts are not significantly different (P < 0.05).

Budget: Proposed project duration: Termination of current project. Funding 1999-2001: \$154,335 Current year request: \$0